

Effect of naloxone treatment on luteinizing hormone and testosterone concentrations in boars with high and low libido

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SUMMARY

The objective was to determine the effects of the opioid peptide receptor antagonist, naloxone on circulating concentrations of luteinizing hormone (LH) and testosterone in boars characterized as having high (n=8) or low libido (n=8) based on the willingness to mount an artificial sow and allow semen collection. On the day of the experiment, blood was sampled every 15 min for 4 h before and 4 h after i.v. injection of naloxone (1 mg/kg body weight). After naloxone treatment, a libido status by time interaction was detected and concentrations of LH within 15 min after treatment were greater ($p < 0.05$) for High-libido boars than for Low-libido boars. Concentrations of testosterone were highly variable amongst boars and there were no effects of libido status ($p = 0.66$) or libido status by time ($p = 0.66$). There

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was, however, an effect of time ($p \leq 0.01$), and concentrations of testosterone in samples collected between 0.5 and 1.25 h after naloxone were greater than concentrations in samples collected prior to injection. In summary, the responsiveness of the hypothalamic-gonadotropic-gonadal axis to opioid receptor antagonism was heightened in boars displaying a high level of sexual motivation. *Reproductive Biology* 2009 9 3: 241-252.

Key words: opioids, luteinizing hormone, testosterone, sexual behavior, boars

INTRODUCTION

As in other mammals, gonadotropin-releasing hormone (GnRH) is episodically secreted from the median eminence in swine, traveling *via* the hypothalamic-hypophysial portal vasculature to the anterior pituitary gland, where it stimulates synthesis and secretion of the gonadotropins, namely luteinizing hormone (LH) and follicle stimulating hormone (FSH; [6]). In boars, LH and FSH stimulate spermatogenesis and secretion of testicular steroids responsible for maintaining libido and controlling gonadotropin release *via* classical negative feedback mechanisms.

The central discharge of GnRH is tonically inhibited by the opioid peptide neuronal system and treatment of mature boars with naloxone (1 mg/kg body weight) increased LH secretion [10]. Naloxone competitively antagonizes μ -opioid receptors in the central nervous system. The drug acts rapidly and has a half-life of 1 to 1.5 h; naloxone is metabolized by the liver and excretion is by urine and bile. In rams, the pattern and magnitude of the LH and testosterone responses to naloxone treatment differ for sexually active and inactive individuals during the breeding season, and these endocrine changes have been proposed as a test for predicting libido [19]. It has not been determined if the LH and testosterone responses to naloxone treatment differ for boars displaying differing levels of sexual behavior. If so, then perhaps a prospective test could be developed for predicting how quickly boars can be trained for semen collection. This test could be used as a tool to assure that only boars with a reasonably good chance of being successfully trained

are selected for entry into artificial insemination breeding programs. Thus, the objective of the experiment reported herein was to determine the effects of naloxone treatment on circulating concentrations of LH and testosterone in boars characterized as having high or low libido.

MATERIALS AND METHODS

The experiment was conducted in June, 2005 at the Virginia Tech-Tide-water Agricultural Research and Extension Center in Suffolk, VA, USA and the protocol was approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University. A total of 39 Yorkshire×Landrace boars were housed in a curtain-sided building in individual pens (4.5 m²) that had a combination of concrete and solid steel rod flooring. Each pen was fitted with a nipple waterer and boars were floor-fed at a rate of 2.27 kg/d a fortified corn and soybean meal-based diet that met or exceeded NRC [16] nutrient recommendations for mature boars.

Boars were moved at least twice weekly to an isolated pen equipped with an artificial sow in an attempt to train the animals for semen collection by the gloved hand technique. All training sessions and semen collections were performed by the same trained technician. After 4 wk, 31/39 boars (79%) had been successfully trained (i.e., upon entry to the collection pen, readily mounted the artificial sow and allowed collection of a complete ejaculate). Eight of these trained boars were randomly chosen for use in the experiment and were classified as High-libido boars. The eight boars that were not successfully trained were classified as Low-libido boars; these individuals typically displayed little or no interest in the artificial sow upon entry into the collection pen and consistently failed to mount. Body weight and backfat thickness at the tenth rib (determined using A-mode ultrasonography; Sono-grader, Renco Inc., Minneapolis, MN, USA) were 171.8±4.3 kg and 10.5±0.8 mm, and 169.2±4.3 kg and 9.4±0.8 mm for the High- and Low-libido boars, respectively ($p>0.3$; $n=8$ per group). At the time of the experiment boars were 368.9±1.2 d of age and all were clinically healthy.

Twenty-four h prior to the experiment, boars were briefly restrained with a metal hog snare and fitted with indwelling jugular vein catheters [13]. On the day of the experiment, blood was sampled every 15 min for 4 h before and 4 h after i.v. injection of naloxone hydrochloride (1 mg/kg body weight; N-7758; Sigma-Aldrich Co., St Louis, MO, USA). The dose of naloxone was previously shown to elicit LH secretion in mature boars [10] and sequential blood samples were collected in order to assess LH pulse characteristics.

Collected blood samples were allowed to clot overnight at 4°C. Serum was harvested following centrifugation and stored at -20°C until assayed. Samples were quantified for LH by radioimmunoassay as previously described [12]. Sensitivity of the LH assay was 0.15 ng/ml and intra- and inter-assay coefficients of variation were 2.8% and 0.9%, respectively. Testosterone concentrations were determined using a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA) validated for porcine serum [1] and that had a sensitivity of 0.04 ng/ml.

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). Mean serum LH concentrations, LH pulse frequency, LH pulse amplitude, and mean serum testosterone concentrations were determined for the 4-h periods before and after naloxone treatment for each boar and subjected to repeated measures analysis of variance. The model included libido status (High or Low), boar within libido status, period (before or after naloxone), and the libido status by period interaction as possible sources of variation. Libido status was tested by boar within libido status and period was tested using the libido status \times period interaction as the appropriate error term. An LH pulse was defined as an increment exceeding twice the standard deviation associated with the mean LH concentration of a serum pool that was analyzed eight times in each assay and pulse amplitude equaled the difference between the pulse peak and the preceding nadir [7].

For the 4-h interval following treatment with naloxone, LH and testosterone concentrations in individual samples were subjected to analysis of variance for a repeated measures design. The model included libido status, boar within libido status, time, and the libido status by time interaction as possible sources of variation. Libido status was tested by boar within libido status and time was tested using the libido status \times time interaction as the er-

ror term. For these analyses, mean LH and testosterone concentrations for the 4-h period prior to naloxone administration for each boar was defined as the time 0 sample. Individual means were compared using the PDIFF option of the GLM procedure of SAS.

RESULTS

Table 1 contains mean concentrations of LH and testosterone in serum, and LH pulse frequency and amplitude for the High- and Low-libido boars for the 4-h periods before and after naloxone administration. Prior to naloxone treatment, mean serum LH and testosterone concentrations and LH pulse frequency were similar for High- and Low-libido boars. The amplitude of LH pulses, however was greater ($p < 0.05$) in Low-libido, compared to High-libido boars. Mean LH and testosterone concentrations were not affected by libido status, period (before or after naloxone) or libido status by period ($p > 0.18$). The frequency of LH pulses tended to decrease after naloxone treatment in High-libido boars, but was unaffected by the opioid antagonist in Low-libido boars (libido status by period interaction, $p = 0.08$). The amplitude of LH pulses decreased after naloxone treatment in Low-libido boars but was unchanged by naloxone treatment in High-libido boars (libido status by period interaction, $p \leq 0.05$).

Profiles of concentrations of LH and testosterone for boars during the 4-h period after injection of naloxone are shown in Figures 1 and 2, respectively. There were no effects of libido status ($p = 0.51$) or time ($p = 0.69$) for LH. There was, however, a libido status by time interaction detected ($p < 0.01$; fig. 1). Concentrations of LH were greater ($p < 0.05$) for High- libido boars than for Low-libido boars within 15 min after naloxone treatment. In contrast, LH concentrations tended to be greater ($p < 0.07$) for Low-libido boars, compared to High-libido boars, between 2 and 2.5 h, and 3.25 and 3.5 h after naloxone treatment.

Concentrations of testosterone were highly variable amongst boars and there were no effects of libido status ($p = 0.66$) or libido status by time ($p = 0.66$). There was, however, an effect of time ($p \leq 0.01$), and concentra-

Table 1. Characteristics of luteinizing hormone (LH) and testosterone for the periods before and after treatment with naloxone in boars characterized as having high or low libido¹

Item:	High-libido (n=8)		Low-libido (n=8)		SEM	p-values		
	Before	After	Before	After		Libido status	Period	Libido status × period
Mean LH (ng/ml serum)	0.39	0.36	0.48	0.41	0.02	0.37	0.27	0.39
LH pulses/h	0.94 ^a	0.72 ^b	0.84 ^{ab}	0.93 ^a	0.08	0.55	0.76	0.08
LH pulse amplitude (ng/ml serum)	0.23 ^c	0.28 ^c	0.40 ^d	0.29 ^c	0.04	0.21	0.78	0.05
Mean testosterone (ng/ml serum)	10.57	11.98	8.97	9.75	1.29	0.66	0.18	0.81

¹Values are means for samples collected at 15-min intervals for 4 h before and 4 h after i.v. naloxone (1 mg/kg body weight) administration; ^{a,b}means with different superscripts tend to differ ($p < 0.08$), ^{c,d}means with different superscripts differ ($p < 0.05$)

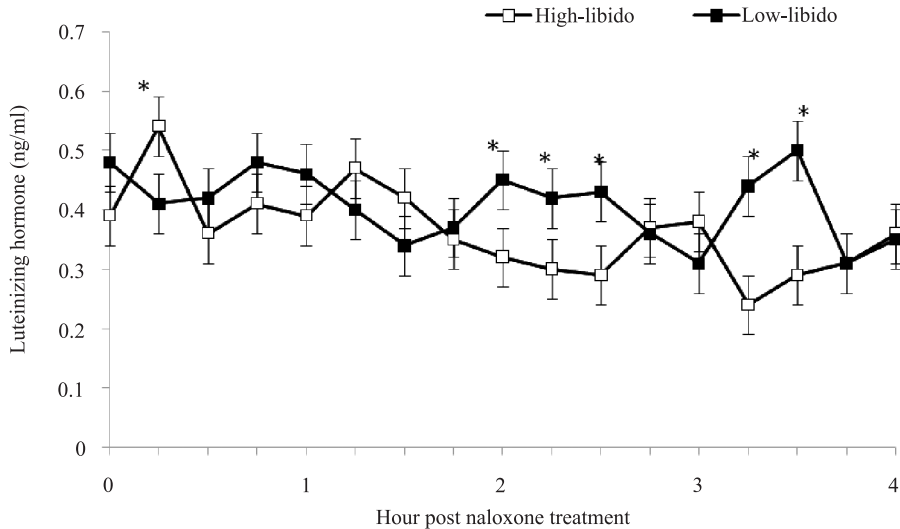


Figure 1. Luteinizing hormone concentrations (means \pm SEM) in serum of High- and Low-libido boars ($n=8$ /group) treated with naloxone. Blood samples were collected at 15-min intervals for 4 h after naloxone. The time 0 samples represent the mean of concentrations in samples collected at 15-min intervals for 4 h before naloxone. There was a libido status by time interaction ($p<0.01$). Within times, means that differ or tend to differ between High- and Low-libido boars are indicated by an asterisk (*; $p<0.01$ to $p=0.07$).

tions of testosterone in samples collected at 0.5 h (12.6 ng/ml; $p<0.05$), 0.75 h (13.0 ng/ml; $p\leq 0.01$), 1 h (13.8 ng/ml; $p<0.01$), and 1.25 h (12.0 ng/ml; $p=0.09$) after naloxone treatment and pooled across libido status were greater or tended to be greater than concentrations in the 0 h sample (9.8 ng/ml) pooled across libido status.

DISCUSSION

In boars, as in males of other mammalian species, LH stimulates release of testosterone [6], and it is clear that this testicular steroid plays a critical role in maintaining sexual behavior [11]. Sexual behavior decreased by 30 to 60 d after castration in mature, sexually active boars, and was restored

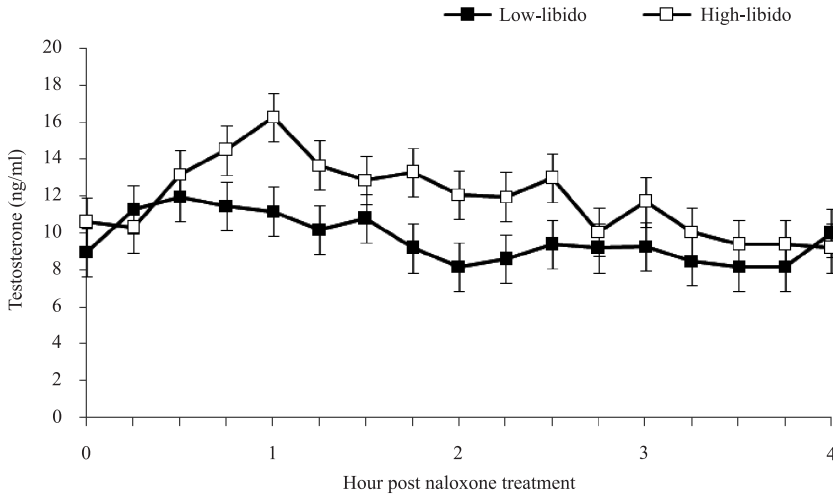


Figure 2. Testosterone concentrations (means \pm SEM) in serum of High- and Low-libido boars (n= 8/group) treated with naloxone. Blood samples were collected at 15-min intervals for 4 h after naloxone. The time 0 samples represent the mean of concentrations in samples collected at 15-min intervals for 4 h before naloxone. There was an effect of time ($p\leq 0.01$) but no effects of libido status ($p=0.66$) or libido status by time ($p=0.66$).

by exogenous treatment with testosterone [14]. It is apparent that a threshold circulating concentration of testicular steroids is required for maintenance of libido and provided this threshold is achieved, the degree of sexual motivation is unrelated to the concentration of testosterone [11]. For example, treating boars with a GnRH agonist dramatically reduced circulating concentrations of testosterone and estradiol (presumably by pituitary LH receptor desensitization and down regulation) but was largely ineffective in suppressing libido [9]. In the current investigation, prior to naloxone treatment, mean testosterone concentrations were similar for boars that were readily trained to mount an artificial sow and allow semen collection and boars that displayed no interest in doing so. It therefore appears likely that the lack of sexual motivation displayed by boars in this experiment was not due to inadequate basal testosterone concentrations but to some other

undetermined cause. Indeed, genetics, season, and rearing environment, as well as sexual and psychological factors, can affect sexual behavior in males [11]. Moreover, estradiol concentrations were not determined in samples collected during this experiment. Blood levels of estradiol increase with age in boars [8] and are inversely related to the time required to mount and begin ejaculation once boars are in the presence of an artificial sow [15]. Estradiol concentrations were higher in boars that readily mounted an artificial sow than in boars that displayed a reluctance to do so [15].

That opioid peptides tonically inhibit LH secretion in female swine is well established. For example, central administration of β -endorphin [17] and morphine [7] decreased LH release in ovariectomized gilts. In contrast, treatment with the opioid receptor antagonist naloxone increased LH release in gilts and sows [2, 3, 4].

The role of opioids in controlling gonadotropin secretion in boars has been less studied and is less clear. In immature boars, systemic injections of morphine failed to alter LH secretion [20], as did treatment with naloxone [21]. However, in an experiment conducted by Patchev *et al.* [18], naloxone stimulated LH secretion in immature barrows that were implanted with testosterone, but not in control barrows or similar-aged boars that received no exogenous steroids. Those authors hypothesized that the achievement of adult levels of testosterone was necessary to activate naloxone sensitive neuronal pathways that tonically inhibit GnRH, and hence LH secretion. Consistent with this hypothesis, Estienne *et al.* [10] reported that treatment of mature boars with naloxone at a dose of 1 mg/kg body weight increased circulating concentrations of LH.

Barb *et al.* [5] proposed that opioid inhibition of LH secretion in gilts is the culmination of a maturational process occurring in the central nervous system. In that study, naloxone treatment stimulated LH secretion in ovariectomized gilts treated with progesterone, but failed to alter release of LH in prepubertal gilts or prepubertal gilts administered progesterone. However, when prepubertal gilts that were ovariectomized and later given progesterone therapy at an age at which contemporary gilts had reached puberty, naloxone treatment readily evoked LH release. Perhaps a similar maturational system exists in the boar.

To our knowledge, this is the first report of research conducted to determine if responsiveness of the hypothalamic-pituitary-gonadal axis to naloxone treatment differed for boars that displayed different levels of libido. Concentrations of LH after naloxone treatment were greater for High-libido boars, compared to Low-libido boars within 15-min after treatment. And although testosterone concentrations increased in both groups following naloxone (effect of time, but no effect of libido status or libido status by time interaction), visual inspection of the data (fig. 2) revealed that concentrations were numerically greater for the High-libido boars at most sample times. Greater concentrations of LH displayed in Low-libido, compared to High-libido boars, at 2 to 2.5 h, and 3.25 to 3.5 h post-naloxone treatment, could be indicative of an escape from negative feedback caused by a lesser increase in testosterone release. Our finding that LH pulse frequency decreased after naloxone treatment in High-libido, but not Low-libido boars is consistent with this hypothesis.

Stellflug et al. [19] reported a series of experiments, the objectives of which were to determine whether LH and testosterone respond differently to naloxone injection in relation to varying sexual performance in rams, and to determine whether differences could predict variation in sexual performance. In 2.5 yr-old rams, a sexual activity by time interaction for both LH and testosterone was related to an earlier increase of both hormones for sexually active rams than the increase displayed by inactive rams. Moreover, sexually active rams had a greater increase in testosterone than inactive rams. The greatest prediction accuracy was 85% using LH at 15 min multiplied by testosterone at 60 min after naloxone treatment. Whether or not LH and testosterone responses to naloxone could be used as a test to predict various aspects of sexual behavior in boars, such as the rapidity with which the animals can be trained to mount an artificial sow and allow semen collection will require further experimentation.

In summary, LH secretion, and probably testosterone release as well, was increased more dramatically by naloxone treatment in High-libido versus Low-libido boars. The results of this study are consistent with the notion that the responsiveness of the hypothalamic-gonadotropic-gonadal axis to opioid receptor antagonism is heightened in boars displaying a high level of sexual motivation.

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